Amendments to the Specification:

Please add the following new paragraph at page 1, prior to the section entitled "GOVERNMENT SUPPORT" the following:

--CROSS REFERENCE TO RELATED APPLICATIONS

This application is a divisional of U.S. Application serial number 09/786,066, filed February 28, 2001, which is the U.S. National Stage of International Application No. PCT/US99/19434, filed on August 30, 1999, which claims the benefit of U.S. Provisional Application serial number 60/098,598, filed on August 31, 1998, the complete disclosures of each are expressly incorporated herein by reference in their entirety.--

Please replace the paragraph beginning at page 1, line 24, with the following amended paragraph:

-- Existing methods used for determining polymer [forma-tion] formation and degradation within cells or organisms are often referred to as "metabolic labeling" [tech-niques] techniques. By far the most common current technique for the in vitro metabolic labeling of cells or tissues in culture uses [[35S]] ^{35}S-methionine as a probe to measure the formation or degradation of cellular proteins. The technique of using radioactively-labeled amino acids as metabolic probes dates back to the late 1940's. (Tarver, *et al.*, *J. Biol. Chem.* 167:387-394 (1947)). --

Please replace the paragraph beginning at page 2, line 17, with the following amended paragraph:

-- Use of a stable isotope for analyzing incorporation of a probe into biopolymers during in vivo metabolic studies has several problems. These problems include but are not limited to: 1) the need to breakdown the biopolymer of interest into smaller components (often amino acids); 2) the need to chemically derivatize the components followed by separating the components within their classes (often by [gas-chro-matography] gas chromatography); and 3)

analyzing the mass of each component using a mass spectrometer. (Halliday and Read, *Proc. Nutr. Soc.* 40:321-334 (1981)). --

Please replace the paragraph beginning at page 3, line 24, with the following amended paragraph:

-- The present invention provides a non-radioactive technique for determining polymer formation or degradation, rapid processing and measurement of a large number of different polymers. In one aspect, the method includes adding a mass isotopically labeled [compo-nent] component of a polymer (probe) to a system in which the unlabeled component of the same type as the probe has been depleted. Depleting the cellular pool of unlabeled component prior to adding the labeled probe increases the likelihood that during polymer formation, the labeled probe is incorporated into the new polymer. Over a period of time, the mass isotopically labeled probe will be incorporated into the new polymer formed, and the total pool of that polymer is the sum of the polymer present prior to adding the probe and newly formed polymer which has incorporated the probe. --

Please replace the paragraph beginning at page 4, line 11, with the following amended paragraph:

-- The present invention also provides a method for storing experimental data within a searchable database to determine the identity of an individual polymer within a complex of several different polymers. Initially, an individual polymer can be separated from the complex of polymer, if desired, cleaved into smaller fragments, and the mass of the polymer or resultant fragments determined by an analytical [instru-ment] instrument such as a mass spectrometer. For each parent polymer, a specific set of fragments having different masses is generated during the fragmentation procedure, but only a subset of these fragments is found within the mass spectra from the analytical instrument. The set of fragments of specific sizes that is measured by the analytical instrument can be used as a "fingerprint" to identify the parent polymer from which the fragments are derived. The database contains "fingerprints" for a large

number of different polymers, and is used to decipher the individual components of a complex comprising a number of polymers. --

Please replace the paragraph beginning at page 6, line 20, with the following amended paragraph:

-- Fig. 2 depicts a diagram demonstrating the theory behind the determination of biopolymer synthesis using stable isotopes. Shown is a theoretical peptide from a protein with the most abundant peak (the monoisotopic peak) set at 100, the first isotopic peak is set at 75, the second at 50, and the third at 25. (The sum of the peak heights equals 250). A theoretical peptide containing the identical amino acid sequence but with one of the amino acids fully substituted with a [[15N]] ^{15}N will essentially give an identical isotope spectra shifted one mass unit higher. Mixing a 50% solution of the unsubstituted peptide with a 50% solution of the substituted peptide will give an isotope spectra which is a mixture of the two spectra found for the individual peptides. Normalizing the monoisotopic peak to 100, and all other peaks at this spectra based on a monoisotopic peak will give a sum of peak heights equal to 500. The percent of peptide containing probe within the mixture can be determined using equation 1. --

Please replace the paragraph beginning at page 6, line 32, with the following amended paragraph:

-- Figs. 3A-3C depict the mass peak distribution for the mouse actin peptide SYELPDGQVITIGNER (SEQ ID NO: 1) Fig 3A depicts the peak mass distribution for the peptide from a control sample. Fig. 3B depicts the mass peak distribution for the peptide from a sample grown in the presence of [[15N]] ^{15}N-isoleucine and [[15N]] ^{15}N-leucine for 24 hours. Fig. 3C depicts the mass peak distribution for the peptide from a sample grown in the presence of [[15N]] ^{15}N -leucine for 53 hours (the peptide contains 2 isoleucine residues and one leucine). for all three panels, the spectra shown have normalized sums of the ion peaks which were virtually identical to the means of the sums calculated for all of the spectra analyzed for the peptide (control, n=23; 24 hrs., n=17; 53 hrs., n=12). --

Please replace the paragraph beginning at page 7, line 26, with the following amended paragraph:

-- The "isotopomeric masses" of a polymer or fragments thereof are all of the [differ-ent] different combinations of the polymer or fragment in the presence of any naturally occurring stable isotope of the elements making up the polymer or a fragment thereof. --

Please replace the paragraph beginning at page 9, line 4, with the following amended paragraph:

-- As used in the present invention, mass isotopically labeled components, or monomer subunits, can be distinguished from naturally occurring, non-labeled components, by being one mass unit heavier. The stable isotopes of common elements useful in the methods of the present invention include, but are not limited to, carbon (^{13}C), hydrogen (^{2}H), oxygen (^{18}O), and nitrogen (^{15}N). These stable isotopes are available commercially as elements or incorporated into components of a biopolymer. In particular, ^{15}N-labeled basic sugars (such as, glucose), amino acid residue components of proteins [basic sugars, such as glucose], and the like, are available commercially. In the practice of the present invention it is preferred that the labeled element or component be stable, and that any preparation containing the labeled component be at least more than about 85%, typically more than 95%, and preferably more than about 98% enriched with the stable isotope or isotopes. A high level of enrichment is preferred to ensure that the majority of the component monomer incorporated into newly synthesized polymer is labeled. --

Please replace the paragraph beginning at page 13, line 19, with the following amended paragraph:

-- The abundance of the monoisotopic and isotopomeric peaks can be expressed in a number of ways, but is typically expressed by scoring the highest or most abundant peak as 100%. All other peaks within the mass spectra for the polymer or fragment thereof are compared to the most abundant peak. To determine the relative abundance of each mass peak, peak heights or the area under the curve can be used. In unlabeled samples, and for large polymers or

polymer fragments, the most abundant peak measured is usually found to be the first or second isotopomeric peak rather than the [monoisotopomeric] monoisotopic peak because of the incorporation of more than one labeled component [monomers] monomer. For the purposes of the present invention, typically the whole spectra of monoisotopic and isotopomeric peaks determined from a labeled sample are compared to the whole spectra from an unlabeled sample. --

Please replace the paragraph beginning at page 22, line 27, with the following amended paragraph:

-- Resuspended cells were transferred to a 1.5 ml screw-cap tube containing 0.28 g of 0.5 mm glass beads, and vortexed vigorously for 2 minutes. The cell/glass bead mixture was centrifuged at 5000 xg for 10 seconds at 4°C. The liquid was withdrawn and transferred to prechilled 1.5 ml tubes containing 0.1 volumes (about 7 ml) or DNase/RNase mixture (1.0 mg/ml DNase I, 0.5 mg/ml RNase A, 50 mM $MgCl_2$, 50 mM Tris-HCl, pH 7.0). The mixture was incubated on ice for 2 minutes. Typically about 70 ml out of 100 ml was recovered. An equal volume of 2X solubilization buffer (the 2X solution contains 0.6% SDS, 2% β-mercaptoethanol, 0.1 M Tris-HCl, pH 8.0) was added, and the tubes were plunged [tube] into boiling water and incubated for 1 minute. All subsequent steps in the sample preparation were identical to those described above for mammalian cell preparation. --

In Table 2 on page 30, please replace column 1, row 2 with the following:
-- AVFPSIVGR (SEQ ID NO. 3) --

In Table 2 on page 30, please replace column 1, row 3 with the following:
-- DLTDYLMK (SEQ ID NO. 4) --

In Table 2 on page 30, please replace column 1, row 4 with the following:
-- AVFPSIVGRPR (SEQ ID NO. 5) --

In Table 2 on page 30, please replace column 1, row 5 with the following:

-- SYELPDGQVITIGNER (SEQ ID NO. 1) --

In Table 2 on page 30, please replace column 1, row 6 with the following:

-- DDDIAALVVDNGSGMCK (SEQ ID NO. 7) --

In Table 2 on page 30, please replace column 1, row 7 with the following:

-- VAPEEHPVLLTEAPLNPK (SEQ ID NO. 8) --

In Table 2 on page 30, please replace column 1, row 8 with the following:

-- DLYGNVVLSGGFTMFPGIADR (SEQ ID NO. 2) --

In Table 2 on page 30, please replace column 1, row 9 with the following:

-- AGFAGDDAPR (SEQ ID NO. 10) --

In Table 2 on page 30, please replace column 1, row 10 with the following:

-- GYSFTTTAER (SEQ ID NO. 11) --

In Table 2 on page 30, please replace column 1, row 11 with the following:

-- VATVSLPR~ (SEQ ID NO. 12) --

In Table 3 on page 31, please replace column 1, row 2 with the following:

-- LDLAGR (SEQ ID NO. 13) --

In Table 3 on page 31, please replace column 1, row 3 with the following:

-- IIAPPER (SEQ ID NO. 14) --

In Table 3 on page 31, please replace column 1, row 4 with the following:

-- AVFPSIVGR (SEQ ID NO. 3) --

In Table 3 on page 31, please replace column 1, row 5 with the following:
-- QEYDESGPSIVHR (SEQ ID NO. 15) --

In Table 3 on page 31, please replace column 1, row 6 with the following:
-- SYELPDGQVITIGNER (SEQ ID NO. 1) --

In Table 3 on page 31, please replace column 1, row 7 with the following:
-- VAPEEHPVLLTEAPLNPK (SEQ ID NO. 8) --

In Table 3 on page 31, please replace column 1, row 8 with the following:
-- DLYGNVVLSGGFTMFPGIADR (SEQ ID NO. 2) --

In Table 3 on page 31, please replace column 1, row 9 with the following:
-- AGFAGDDAPR (SEQ ID NO. 10) --

In Table 3 on page 31, please replace column 1, row 10 with the following:
-- GYSFTTTAER (SEQ ID NO. 11) --

In Table 3 on page 31, please replace column 1, row 11 with the following:
-- VATVSLPR** (SEQ ID NO. 12) --

In Table 4 on page 32, please replace column 1, row 2 with the following:
-- LDLAGR (SEQ ID NO. 13) --

In Table 4 on page 32, please replace column 1, row 3 with the following:
-- HAPPER (SEQ ID NO. 14) --

In Table 4 on page 32, please replace column 1, row 4 with the following:

-- AVFPSIVGR (SEQ ID NO. 3) --

In Table 4 on page 32, please replace column 1, row 5 with the following:
-- QEYDESGPSIVHR (SEQ ID NO. 15) --

In Table 4 on page 32, please replace column 1, row 6 with the following:
-- SYELPDGQVITIGNER (SEQ ID NO. 1) --

In Table 4 on page 32, please replace column 1, row 7 with the following:
-- VAPEEHPVLLTEAPLNPK (SEQ ID NO. 8) --

In Table 4 on page 32, please replace column 1, row 8 with the following:
-- DLYGNVVLSGGFTMFPGIADR (SEQ ID NO. 2) --

In Table 4 on page 32, please replace column 1, row 9 with the following:
-- AGFAGDDAPR (SEQ ID NO. 10) --

In Table 4 on page 32, please replace column 1, row 10 with the following:
-- GYSFTTTAER (SEQ ID NO. 11) --

Please replace the paragraph beginning at page 33, line 8, with the following amended paragraph:

-- The branched amino acids leucine, isoleucine and valine are known to undergo transamination reactions (Matthews et al., Science 214:1129-1131, 1981). During the reaction, the amino terminus nitrogen from these amino acids can be transferred to a glutamic acid. Peptide 1133 contains 1 glutamic acid residue, and transamination may explain stable isotope incorporation into this peptide. The metabolic labeling of peptide 976.5 AGFAGDDAPR (SEQ ID NO. 10), was unexpected, as the arginine residue found in human actin has been reported to

be converted to a leucine residue in mouse melanoma cells (Sadano et al., J. Biol. Chem. 263:15868-15871, 1988). However, if that were the case, the peptide would no longer have a MW of 976.5, since it would not be cleaved at the same location by trypsin. It is possible that one of the aspartic acids (MW 115.0) may be substituted with a leucine or isoleucine (MW 113.0) in the cell line used. In order to contravene the issue of potential loss of the label due to transamination, the mouse T-cell line can be cultured using ^{15}N-lysine substituted amino acids (see Table 4). --

Please replace the paragraph beginning at page 34, line 9, with the following amended paragraph:

-- The theoretical distribution of isotope peaks for any peptide for which the amino acid sequence or elemental composition is known can be calculated using a set of mathematical formulas (Beynon, In Mass Spectrometry and its applications to organic chemistry, Elsevier Publishing Co., New York, p.294-301, 1960; McCloskey, Meth. Enzymol. 193:882-886, 1990), or by using computer programs such as MS-Isotope (UCSF Mass Spectrometry Facility's MS-Isotope program can be found at [http://prospector.ucsf.edu/ucsfhtml/msiso.htm] the University of California San Francisco ProteinProspector website). For different peptides from control samples, the relative distributions of isotope peaks determined experimentally using MS-zoom scans or MALDI was compared to the theoretical distribution determined by the computer program MS-Isotope. Isotope peak distributions calculated from ion-trap MS data were similar to theoretical distributions for most peptides studied which had mass/charge values of +2 on the MS/ MS (Table 5). On average, MS-Zoom values were 1.08 times higher than theoretical values, while MALDI values were 1.14 times higher than theoretical values. Since the discrepancies between the ratios (53H/24H) determined from ion-trap and MALDI were potentially due to differences in the control isotope peak spectra alone, % substitution levels were calculated using theoretical isotope peak spectra for both ion-trap and MALDI data. Using theoretical control spectra and experimental spectra for the 24 hours and 53 hours time-points, calculated ratios for ion-trap MS were 1.65 ± 0.10, compared to 1.57 ± 0.12 for MALDI. Thus,

it may be possible to use theoretical distributions of isotope peaks for calculations of substitution levels, rather than analyzing multiple control samples. --

Please replace the paragraph beginning at page 36, line 8, with the following amended paragraph:

-- To test whether unmatched peptides were modified peptides from the unknown protein, the masses of the peptides and the identity of the protein analyzed were entered into EXPASY's FindMod (Find Modification) tool found at [http://expasy.hcuge.ch/sprot/findmod.html] the Expert Protein Analysis System of the Swiss Institute of Bioinformatics, a proteomics server website. The program gives a number of possible modified peptides based on the mass of the peptide entered. Using MS/MS data, the identity of the peptide many times could be determined. For example, peptide DLTDYLMK (SEQ ID NO. 4) (MW 998.5) was found to have an oxidized methionine residue and a MW of 1014.5 (Table 2). In addition to the presence of a carboxyamidomethyl cysteine (+58) formed when the first dimension strip was treated with iodoacetamide, peptide DDDIAALVVDNGSGMCK (SEQ ID NO. 7) (MW 1722.8) was found to be acetylated (+42) at the N-terminus, while the methionine residue was either unoxidized, singly (+16), or doubly (+32) oxidized giving experimental MW's of 1823.8, 1838.6, and 1855.6. Thus, the stable isotope metabolic labeling technique was found to be useful in helping to select peptides from MS scans which may potentially be modified.